

Metabolic Activation of Toxins: Tissue-specific Expression and Metabolism in Target Organs

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Cytochrome P450 (CYP) enzymes catalyze the generation of reactive species capable of binding with cellular macromolecules, leading to acute and delayed toxicity. Since individual CYP forms differ markedly in their substrate preferences and regulation, the expression profiles of CYP in various cell types are important determinants in tissue-specific toxicity. The highest concentrations of most forms of CYP are found in liver, but they are also present in many extrahepatic organs. Liver is also a target organ in which CYP-mediated activation and toxic outcome have been most convincingly linked. Prime examples are paracetamol-induced hepatotoxicity and aflatoxin B₁-associated hepatic cancer. In contrast to liver, most extrahepatic tissues are composed of multiple cell types, which make experimental approaches difficult. Also the low abundance of individual forms is a challenge in the study of extrahepatic CYP-related toxicity. Recent years have witnessed the emergence of molecular biological techniques, e.g., reverse transcriptase-polymerase chain reactions, which facilitate the study of low abundant CYP forms in human tissues. Nevertheless, in the end we need definite information on the expression of activity, and for this purpose enzyme-specific substrates, reactions, and inhibitors and other methods to detect proteins and associated activities are needed. In humans, it is important to measure activities of specific enzymes *in vivo*. For this purpose, two approaches are currently available. Metabolism and/or elimination of enzyme-specific drugs can be employed. In cases in which genetic background determines the presence or absence of a specific enzyme, phenotyping and genotyping tests can be devised, e.g., for CYP2D6 (debrisoquine hydroxylation) polymorphism. — *Environ Health Perspect* 105(Suppl 4):767–774 (1997)

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Introduction

The concept of metabolic activation, i.e., formation of reactive metabolites, has been a very useful paradigm in chemical carcinogenesis and chemical-induced toxicity for three major reasons: first, it has provided a mechanistic basis for understanding the initial action of structurally diverse carcinogens and toxicants; second, it has provided an explanation for the binding of many chemical compounds to DNA and

other macromolecules; and third, it has been a crucial bit of knowledge in the creation of short-term genotoxicity and other toxicity tests for the prediction of toxic reactions, including chemical carcinogenesis. Although this paradigm is overwhelmingly accepted, the exact details are often lacking. For example, most studies on metabolic activation have been conducted with liver preparations with an abundant

assortment of both activating and inactivating drug-metabolizing enzymes. The expression of crucial enzymes at the site of action, other than liver, has not often been adequately studied.

Formation of reactive metabolites is catalyzed principally by the so-called drug- or xenobiotic-metabolizing enzymes, but some activating reactions are catalyzed also by enzymes principally involved in endogenous metabolism. The most important group of activating enzymes is the large P450 (CYP) "superfamily" (1) catalyzing oxidative (and sometimes reductive) reactions; but practically all conjugating enzymes are also capable of forming reactive metabolites from a few selected substrates, e.g., reactive glutathione conjugates are generated of some halogenated compounds.

The prevailing opinion thus far has been that for a given compound, metabolic activation is rather difficult to predict because it depends on both the structure of the substrate and the specific enzymes involved. Another hindrance in the prediction of consequences of metabolic activation has been the lack of exact knowledge about endogenous and exogenous factors affecting the level and balance of enzymes. However, recent studies on the activation of numerous potent genotoxins and potential carcinogens by human liver enzymes demonstrate that a relatively small number of CYP forms primarily activate genotoxins and other toxicants. Such studies show promise in that it may be possible in the future to predict an individual's ability (even the inherited ability) to activate specific toxins.

One of the crucial endogenous factors is polymorphic variability of various CYP forms (Table 1), which is of considerable clinical importance but which may also be significant in individual susceptibility to chemical carcinogens and other toxic substances. However, the significance of CYP polymorphisms in the expression and metabolic activation of toxic substances in target tissues other than liver has not been elucidated to any great degree although, hypothetically, variant alleles of structural genes should be similarly expressed in all expression-competent tissues.

This article surveys the present status of the field in terms of tissue-specific expression, metabolism of some toxicants, and especially the methodologies that measure their parameters while focusing upon CYP enzymes.

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Abbreviations used: AHH, aryl hydrocarbon (benzo[a]pyrene) hydroxylase; CYP, cytochrome P450; MAO-B, monoamine oxidase B; MTPT, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; MPP⁺, 1-methyl-4-phenyl-pyridinium ion; PAH, polycyclic aromatic hydrocarbon; RFLP, restriction fragment length polymorphism; RT-PCR, reverse transcriptase-polymerase chain reaction.

Table 1. Polymorphisms of human CYP genes.

Gene	Defective allele	Activity	Remarks
CYP1A1	11e462Val	?	Ethnic variation
CYP1A2	?	?	Variable phenotypes
CYP2A6	v1: Leu160His	0	Ethnic variation
	v2: several changes	0	Ethnic variation
CYP2B6	?	?	Low expression
CYP2C9	11e359Leu	Down	Ethnic variation
CYP2C19	3 null alleles	0	Ethnic differences
CYP2D6	Numerous (about 25)	Up, down, 0	Ethnic differences
CYP2E1	5', 3', intron	?	Ethnic variability
CYP2F1	?	?	?
CYP3A4	2 AA changes	?	Rare
CYP3A5	?	0	Incidence unknown
CYP3A7	?	?	?
CYP4B1	?	?	?

Significance of Tissue-specific Expression of CYPs

The potential role of selective presence of CYP forms in different tissues can be derived from many forms that are known to generate reactive metabolites *in situ* from a number of xenobiotics. Thus, selective expression of CYP forms possibly contributes to tissue-specific damage caused by xenobiotics. Figure 1 is a simplified scheme illustrating the two main possibilities. If the metabolite formed in the liver or any other tissue is stable, it can be transported locally or through the bloodstream to distant sites, where it can produce the effects. The other possibility is that the parent compound undergoes metabolic activation in the target tissue itself, and that the reactive metabolites formed attack the macromolecules of the activating cell. Given the highly reactive nature of many oxidized compounds, the

latter possibility appears more plausible in most instances. Nevertheless, examples of the first possibility include monocrotaline-induced lung damage, neurotoxicity caused by tetraethyl lead and *n*-hexane, as well as testis toxicity elicited by 2-methoxyethanol (2). Ultimate toxicity in the target tissue depends on several factors, including the amount of reactive intermediates formed, the extent of detoxication processes, and the efficiency and fidelity of repair systems, such as DNA-repair enzymes.

A heavily studied aspect of tissue distribution of xenobiotic metabolizing enzymes relates to target organ selectivity of chemical carcinogens. A large proportion of the known or suspected carcinogens require metabolic activation; many carcinogens exhibit marked tissue specificity, with a given compound producing cancers at some sites and no effects at others. There is thus a

clear urgency in understanding tissue-specific activation mechanisms. Many human CYP forms are known to be able to activate numerous procarcinogens. The inactivation pathways by CYP and conjugating enzymes are also being unraveled (3–6). The genetic variations in xenobiotic-metabolizing enzymes in relation to cancer have been reviewed in several articles (7–10).

Methods of Studying the Expression of CYP Forms

A large number of chemical, biophysical, biochemical, and molecular biological methods have been used in P450 research. For example, detection of activity, i.e., catalytic capability *in vitro*, uses substrates or reactions with tissue preparations, e.g., lung or liver microsomes and lymphocytes. *In vivo* detection may use probe drugs such as caffeine or debrisoquine and endogenous substances such as 6 β -hydroxycortisol. Detection of expression (message and/or protein) may be accomplished using Western (protein) or Northern blot (mRNA); reverse transcriptase (RT)-polymerase chain reaction in tissue RNA samples, which may be either qualitative or quantitative; *in situ* hybridization; or immunohistochemistry. Detection of genomic status (gene structure, alleles) uses restriction fragment length polymorphism (RFLP) or Southern blot (PCR on DNA). Note that the development of methodologies has made use of liver, with its wide and abundant assortment of P450 and other drug-metabolizing enzymes. The application of many of these methods and approaches to other target tissues faces a number of problems and pitfalls, including the following:

- low amounts and (possible) lability of enzymes
- different profiles of CYP and other enzymes as compared to liver (Table 2)
- unique, tissue-specific enzymes
- tissue-specific regulation of even those enzymes that are expressed in many tissues
- tissue heterogeneity (liver 90% hepatocytes; lungs > 30 cell types) with cell-specific assortments of enzymes
- isolation of cell organelles standardized for liver; not necessarily applicable to other tissues.

Furthermore, after the demonstration of a specific CYP enzyme in a given tissue, we need a variety of approaches to link the activity with the toxic outcome.

There are basically five groups of methods to study the expression of individual CYP

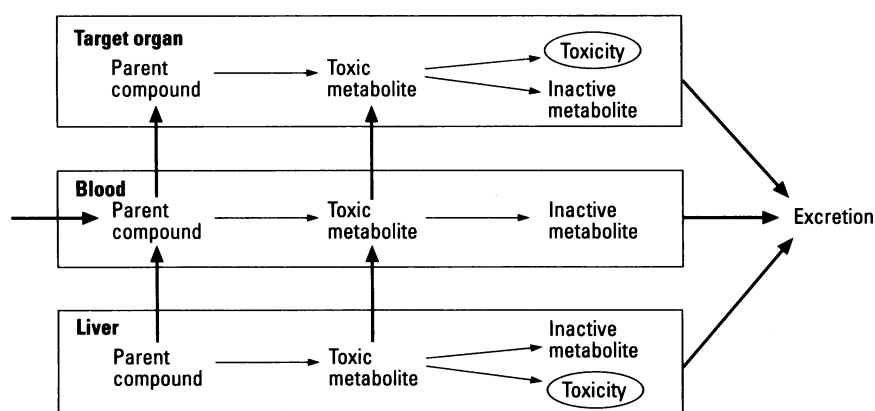


Figure 1. Possible pathways of a xenobiotic and its toxic metabolites in the body. After absorption, the xenobiotic is distributed throughout the body through the bloodstream. The parent compound, if hydrophobic enough, can travel freely in different tissue compartments. Xenobiotic-metabolizing enzymes act in concert to convert the parent compound to a water-soluble metabolite. Toxic metabolites can be formed in virtually any tissue, but the main bulk of metabolites is usually generated in liver. Tissue-specific toxicity depends partially on the amount of metabolizing enzymes present in target tissues. A reactive metabolite can migrate to sites distant from the tissue of origin. For example, reactive metabolites formed in liver can cause toxic effects in lungs.

Table 2. Expression of xenobiotic-metabolizing CYP forms in human tissues.

Organ/tissue	CYP										
	1A1	1A2	2A	2B	2C	2D6	2E1	2F1	3A	4B1	4A11
Liver	++	+++	+++	+++	+++	+++	+++	0	+++	0	0
Lung	+++	ND	ND	+++	ND	ND	++	+++	+++	+++	ND
Small intestine	++	ND	ND	ND	+	+	ND	ND	+++	ND	ND
Skin	++	ND	ND	ND	ND	ND	ND	ND	+	ND	ND
Kidney	+	ND	ND	ND	ND	ND	ND	ND	+++	ND	+++
Brain	++	ND	ND	ND	+	++	+	ND	++	ND	ND
Vascular endothelium	++	ND	ND	ND	ND	ND	++	ND	++	ND	ND
Lymphocytes	+++	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Mammary gland	+++	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Placenta	+++	0	0	0	0	0	+	+	++	+++	ND

ND, not enough data available; +++, strong evidence, based on demonstration of CYP mRNA and/or protein (and often on activity); ++, tentative evidence, based on demonstration of mRNA, catalytic activities or cross-reactions with anti-CYP antibodies, verified in several laboratories; +, preliminary evidence, based on catalytic activities or cross-reactions with antibodies; 0, not present. It is likely that many laboratories have obtained negative data that has not been published, and that many of these isoforms will eventually be found. Modified from Raunio (34).

Table 3. Diagnostic substrates and inhibitors of human CYP forms.

Form	Substrate	Inhibitor
CYP1A1	7-Ethoxyresorufin	7,8-Benzoflavone ^a
CYP1A2	Phenacetin	Furafylline, fluvoxamine
	Caffeine	
CYP2A6	Coumarin	Diethyldithiocarbamate, 8-methoxypsoralen
CYP2B6	6-Aminochrysene	Not known
	Cyclophosphamide	
CYP2C8	Taxol	Quercetin
CYP2C9	Tolbutamide	Sulfaphenazole
CYP2C19	S-Mephenytoin	Tenoposide?
CYP2D6	Debrisoquine, sparteine, dextromethorphan	Quinidine
CYP2E1	Chlorzoxazone ^b	Diethyldithiocarbamate ^c
CYP2F1	Skatole	Not known
CYP3A4	Testosterone, nifedipine	Troleandomycin, gestodene

Compiled from Pelkonen and Breimer (16) and Pelkonen et al. (18). ^aAlso inhibits CYP2C9 and CYP3A4 (66). ^bAlso metabolized by CYP1A1 (67). ^cAlso inhibits CYP2A6 (66).

forms: determination of enzyme catalytic activities in tissue samples using form-selective diagnostic substrates and inhibitors, methods that can also be applied to the *in vivo* situation under certain constraints; purification and reconstitution of CYP proteins from different tissues; utilization of antibodies in various immunodetection protocols; cloning and sequencing of CYP cDNAs and their use as probes in various types of mRNA analysis; and RT-PCR, often combined with the use of specific restriction enzymes, RFLP.

A new and exciting approach is to generate knock-out mice in which specific CYP structural genes or genes encoding regulatory receptor proteins are disrupted

by targeted mutations (11). These mice, lacking the proper function of the disrupted gene, serve as excellent models in the study of the role of these genes in organ development and, if the mutations are compatible with life, also in the various toxicities caused by metabolized xenobiotics (12).

Diagnostic Substrates and Inhibitors

Numerous chemicals have been tested as substrates or inhibitors of CYP forms in experimental animals and humans (13–16). Table 3 lists some of the best established form-specific substrates and inhibitors of human CYPs. Substrates or inhibitors are very seldom, if ever, completely enzyme specific; usually a single substrate can be metabolized by several CYP forms, and a single form can accept several substrates. On the other hand, at low concentrations of several substrates, enzyme kinetics often favor a single form being the primary catalyst of metabolism.

A number of compounds have been found to inhibit P450-mediated metabolism of other chemicals (14,17). Inhibition can be either reversible or irreversible depending upon the chemical. Irreversible inhibitors binding covalently to P450 enzymes are often called mechanism-based or suicide inhibitors. These are often more form specific than reversible ones because the enzyme must initially accept the inhibitor as a substrate, which is then metabolized to a reactive species (13,14).

Currently, with the aid of known enzyme-specific reactions and purified and heterologously expressed human enzymes, it is possible to unequivocally assign relative inhibitor affinities to a series of related enzymes. A selection of inhibitors listed in Table 3 is claimed to express at least a

degree of enzyme specificity, which might be useful in the assignment of metabolism of any new substance to each specific enzyme. Specificity is seldom, if ever, absolute; rather, an inhibitor shows variable affinities to different enzymes. However, for practical purposes, one or two orders of magnitude difference is probably enough for a compound to be useful as a specific or diagnostic inhibitor.

In human studies, for obvious reasons, it would be very useful to have a substance that could be used to measure the activity of an enzyme *in vivo* (a probe drug). However, such an *in vivo* probe has to fulfill more stringent conditions than an *in vitro* substrate (18). Also the validation of an *in vivo* probe, which has to be performed in human subjects—healthy volunteers or patients—is a demanding and cumbersome task and has been accomplished only for a handful of substances. The most thoroughly validated probes are those for polymorphic CYP enzymes (such as debrisoquine or mephenytoin) because they display phenotypically separable, pharmacokinetically defined groups and the correspondence between genotype and phenotype is often relatively direct, e.g., lack of an enzyme shows up as a poor metabolizer.

An example of an *in vivo* probe drug is caffeine; although several distal metabolites are produced, these can be grouped under three enzymes (CYP1A2, NAT2, and xanthine oxidase) and consequently can be used as probes for the respective enzyme. However, despite the wide use of caffeine as an *in vitro* and *in vivo* probe, concern still exists about the uncritical use of caffeine metabolites as probes (19).

P450 Protein Purification

Comprehensive reviews are available about this issue (20,21). Approximately 10 human hepatic CYP proteins in families 1 to 4 have been purified to a degree at which unequivocal form assignment is possible. It is obvious that these forms represent the most abundant ones present in human liver and that forms expressed at low levels in the liver or other tissues cannot be readily purified to homogeneity.

Antibodies and Immunochemical Methods

A large number of polyclonal and monoclonal antibodies have been developed against purified animal and human CYP forms, and many of these are commercially available (21,22). A novel and promising technology is the production of

monospecific antibodies against synthetic CYP peptides (23,24). In general, monoclonal antibodies are epitope specific and thus yield more precise information than polyclonal antibodies. Use of monoclonal anti-CYP antibodies in qualitative and quantitative detection of individual CYP forms has recently been reviewed (21). The advantage of using polyclonal antibodies is that they are usually inhibitory to the target enzyme. Anti-CYP antibodies are used in several techniques as probes to detect the presence of individual CYP forms. The basic methods are catalytic activity inhibition, immunoblotting (Western blotting) and immunohistochemistry. The main advantage of immunohistochemistry is that with this technique the distribution of the detected antigen in tissues can be elucidated. This is an important feature in extrahepatic tissues, which often consist of several parenchymal cell types mixed with supporting cells.

Current immunohistochemical techniques have the sensitivity and resolution to locate specific cell types containing identifiable CYP forms, even in tissues with a low overall P450 content (25). The lung is the best example of a complex tissue in which substantial progress has been made with immunohistochemical approaches in the localization of several distinct CYP forms in various cell types. No other method, excluding *in situ* hybridization, could have yielded such precise information. A comprehensive review on the use of immunohistochemical methods in pulmonary P450 research is available (26).

cDNA Cloning

To characterize the amino acid sequence of almost any protein, the best approach is to determine the nucleotide sequence of its cDNA. cDNA and genomic cloning of CYP forms have made it possible to construct the CYP superfamily (1). The molecular biology of CYPs has been reviewed by Gonzalez (27,28). The cDNAs produced can be employed for various types of mRNA detection protocols, such as the Northern blot and dot-blot methods. *In situ* hybridization, a method that combines the virtues of histochemistry and RNA hybridization techniques, has much potential for studying expression patterns in extrahepatic tissues. The basic steps of this technique are preparation and fixation of tissue slices, treatment to render them permeable to nucleic acid probes, hybridization with radioactive DNA or RNA probes, and autoradiography.

As in immunohistochemistry, the main advantage of *in situ* hybridization is that the distribution of the target molecule in the tissue under study can be evaluated. This is especially helpful in tissues that are composed of several types of cells. This technique is rather laborious and sensitive, and there is no consensus on the optimal handling protocols for human tissues. However, some groups have recently obtained reproducible results with human liver and extrahepatic tissues (29,30).

RT-PCR

The use of PCR-based methods is rapidly increasing in the study of gene expression, and several novel PCR-based methods are now available to measure the amount of specific mRNAs in a quantitative manner (31). The basic protocol to measure gene expression by PCR involves synthesis of cellular mRNA to cDNA with reverse transcriptase, amplification of the cDNA template using gene-specific primers, and detection of the amplification product.

Several groups have used this method to assess the expression of CYP genes in a qualitative manner, i.e., to screen whether a given CYP form is expressed or not in the tissue under study. In the first report in which RT-PCR was used for detection of CYP forms in humans, CYP1A1 message was found in several tissues, including liver and lung (32). Hakola et al. (33) assessed by qualitative RT-PCR the expression of all known CYP forms in families CYP1 to CYP4 in human liver. The results correlated well with known features of CYP expression (Table 2). The RT-PCR approach is being increasingly used to detect CYP form mRNAs in several extrahepatic tissues (34).

Quantitative RT-PCR technique for measuring CYP1A1 expression in human lymphocytes was applied first by Vanden Heuvel et al. (35). An artificial recombinant mRNA (rcRNA) containing the same primer annealing sites as CYP1A1 was used as an internal standard. The rcRNA is added to the cDNA synthesis reaction together with the test RNA, and the amplified products are distinguished by their different sizes in gel electrophoresis. After optimization, this approach yields an absolute quantitation of the amount of CYP1A1 mRNA present in the sample. Treatment of lymphocytes with 10 nM 2,3,7,8-tetrachlorobenzo-*p*-dioxin (TCDD) resulted in a 20-fold increase in the amount of CYP1A1 mRNA, which is very low in uninduced lymphocytes (35).

The PCR methodology is also central in the development of genotyping assays to detect variant alleles of CYP genes. The best validated methods are those that detect the most common variant alleles of CYP2D6 (36,37) and CYP2C19 (38). The latest addition to the genotyping assays is the PCR-based method to detect variant CYP2A6 alleles (39).

Tissue-specific Expression of CYP Forms and Examples of Tissue-specific Toxicity

When we are trying to link metabolic activation and tissue toxicity in a given target tissue, the following prerequisites have to be met:

- The enzymes necessary for the activation have to be expressed in the target tissue, or we have to demonstrate that the reactive intermediate formed in another tissue is transported to the site of action.
- We need to demonstrate that the tissue preparations or isolated cells are able to catalyze the activation reaction.
- We should show that a tissue sample isolated from a human being exposed to a compound under study contains appropriate bound metabolite(s). Alternatively, appropriate animal experiments should give a basis for extrapolation to the human situation, especially if it is not possible to obtain appropriate human tissue samples.
- A logical and biologically plausible sequence of events from exposure, metabolic activation, covalent binding, biochemical changes, and overt tissue damage should be demonstrated.

Naturally, when we are dealing with humans, it is seldom possible to establish an unequivocal sequence of events; but we should try to demonstrate at least some of the necessary factors, e.g., the expression of activating enzyme(s) in the target tissue. A compilation of CYP expression in various tissues is presented in Table 2. Furthermore, some better known examples of chemical-induced tissue toxicities are shown in Table 4. In the next few paragraphs, we summarize examples of CYP expression in potential target tissues.

Liver

Perhaps the most compelling case is provided by paracetamol-induced liver toxicity in which it is possible to construct the logical and plausible sequence of events and also to delineate factors enhancing and opposing toxicity. With most other toxicants,

Table 4. Some examples of CYP-mediated tissue toxicities.

Chemical(s)	Target/reaction	Activating enzyme(s)
Paracetamol	Liver/necrosis	CYP2E1, CYP1A2
Aflatoxin B ₁	Liver/cancer	Multiple
Nitrosamines	Liver/cancer	CYP2E1, CYP2A6?
Haloalkanes	Liver/necrosis	CYP2E1
Tacrine	Liver/toxicity	CYP1A2
4-Ipomeanol	Lung/necrosis	CYP3A
Skatole	Lung/necrosis	CYP2F1
Benzidine	Bladder/cancer	CYP1A2?
Cyclophosphamide	Bladder/cystitis	Multiple
Benzene	Bone marrow/leukemia	CYP2E1
Dapsone	Erythrocytes/methemoglobinemia	CYP3A4

and especially with those causing toxicity in extrahepatic tissues, it is not possible to construct such a logical and well-researched scheme, but with paracetamol-induced toxicity, the following discrete steps can be traced:

- Paracetamol is activated into reactive intermediate (quinoneimine) by CYP2E1, CYP1A2, and CYP3A4 both *in vitro*, *ex vivo*, and *in vivo*.
- In normal doses, main metabolic pathways (glucuronidation, sulfoconjugation) lead to inactive products.
- Active metabolite is inactivated by glutathione, partly catalyzed by glutathione S-transferase.
- Formation of active metabolite is inhibited by known CYP form-specific chemical inhibitors and antibodies, as well as by glutathione precursors or derivatives.
- Activation is enhanced by ethanol and phenobarbital pretreatment.
- Active metabolite is covalently bound to hepatic proteins.
- Several possible and plausible cellular events may lead from covalent damage to overt cellular damage and necrosis.

Another established case is the association between activation and covalent binding of numerous hepatocarcinogens (e.g., aflatoxin B₁) and the development of liver cancer. Relatively good correlation exists between DNA adducts and carcinogenic potency of several hepatocarcinogens. P450 enzyme-specific activation reactions have been elucidated for many of these compounds, making it possible, for example, to predict the influence of appropriate P450 polymorphisms on cancer risk (40,41).

Lung

Pulmonary tissue is the first to encounter all inhaled xenobiotics. Several mechanisms

have developed in the lung to minimize the entry of foreign compounds into the systemic circulation, including the presence of P450 and conjugating enzymes in the various pulmonary cell types. The lung is a target for tobacco smoke-induced lung cancer, one of the best characterized chemical toxicities in humans. Several procarcinogens present in tobacco smoke are, after metabolic activation, capable of inducing and promoting neoplastic transformation in the lung cells (42).

The human lung has an extensive complement of xenobiotic-metabolizing enzymes, the main features of which are the following:

- 100,000×g pellet (microsomal fraction) contains little endoplasmic reticulum
- Total P450 spectrum detectable
- Extensive oxidation and metabolic activation of PAH compounds
- Heterogeneity in CYP expression among different cell types
- CYP1A1 shown at catalytic activity, protein, and mRNA levels
- CYP1A1 inducible by cigarette smoking
- CYP2B7 and CYP3A5 mRNAs abundantly present
- CYP2F1, CYP4B1 and CYP5 cloned from human lung cDNA library
- CYP2E1 and CYP3A shown at protein level

Benzo[*a*]pyrene and other polycyclic aromatic hydrocarbons are activated to DNA-binding metabolites by human lung tissue and catalytic activities mediated by members in the CYP1A subfamily are measurable and inducible in the lungs.

More direct evidence for the presence of cigarette smoke-inducible CYP1A1 in lung was provided by several studies in which CYP1A1 was assessed at the mRNA, protein, and catalytic activity levels (43,44). These studies have also demonstrated that CYP1A1 inducibility is higher in patients with lung cancer than in control subjects. The observed induction is likely to be *Ah* receptor mediated, since the receptor is present in lung (45,46). The CYP1A1 protein capable of oxidizing 7,8-dihydroxy-7,8-dihydrobenzo[*a*]pyrene to a genotoxic product has recently been purified from human lung (6).

An example of selective toxicity based on metabolic activation in the target tissue is lung cell death caused by 4-ipomeanol. This toxin is activated by P450 enzymes in several cell types in the lung, especially the Clara cells, whereas only marginal activation occurs in other tissues (47). Studies with a panel of heterologously expressed

human CYP forms have shown that metabolic activation of 4-ipomeanol is mediated by CYP1A2 and CYP3A4 (48), a finding that fits well with the presence of CYP3A members in the lung (49,50). Another lung toxin, skatole (3-methylindole), is activated by a lung-specific form, CYP2F1 (51).

Brain

Considerable attention has been paid to the role of environmental toxins in the etiology of central nervous system disorders. In particular, exogenous neurotoxins have been suggested to cause Alzheimer's and Parkinson's disease (52). Xenobiotics entering the central nervous system have to be very lipophilic to be able to cross the specialized endothelium of cerebral blood vessels (blood-brain barrier). It is thus conceivable that metabolism in blood vessel endothelial cells, as well as in neuronal and adjacent support cells, would greatly affect the toxicity elicited by the xenobiotics entering the brain.

The role of CYPs in brain may include such diverse functions as metabolism of xenobiotics, aromatization of androgens to estrogens, and formation of catechols; CYPs may also participate in metabolism of neurotransmitters (53). In rodents, brain P450 levels are low, but there exist some hot spots such as olfactory areas, cerebellum, and brainstem where P450 activities are exceptionally high. The same type of localization is found in human brain (54). Demonstration of the presence of P450 in the human brain microvessels and choroid plexuses (55) opens the question of whether metabolism in endothelial cells may regulate the penetration of the xenobiotics to the brain compartment.

Multiple CYP forms are found in the brain of rats (56). Several CYP forms are inducible in the rat brain during pregnancy, lactation, and treatment with P450 inducers, such as ethanol, chlorpromazine, phenobarbital, and nicotine (53). Information about human brain P450 is limited and is mainly based on autopsy material that may not reflect the true *in vivo* situation for a number of reasons. For example, there may be considerable discrepancy in overall P450 levels among different studies. Localization is probably extremely nonhomogeneous, and there may be xenobiotic-metabolizing CYP forms in subfamilies 1A, 2D and 3A. We know that steroid-metabolizing CYP forms are definitively present in subfamilies 11A and 19. There may also be a possible role

for CYPs in neurotoxin metabolism, either in activation or inactivation. Evidence thus far has been fragmentary and not always convincing (34).

An especially intriguing case is cerebral CYP2D6. There are indications for its presence in distinct regions of human brain and involvement in detoxification of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a potent neurotoxin that causes Parkinson's disease-like symptoms. MPTP is oxidized to the 1-methyl-4-phenol-pyridinium (MPP⁺) ion by monoamine oxidase B (MAO-B) in astrocytes and then taken into dopaminergic neurons via the dopamine transporter. CYP2D6, in turn, oxidizes MPTP into a less active metabolite (52,57). Several studies have demonstrated that a CYP2D6-poor metabolizer genotype is connected with a 2- to 3-fold increased risk of developing Parkinson's disease, whereas other studies have not come to the same conclusion (57). The overrepresentation of CYP2D6-poor metabolizers in patients with Parkinson's disease might reflect a reduced capacity to detoxify ingested MPTP-like neurotoxins.

CYP2D6 is also known to metabolize several centrally acting drugs (58,59). It is thus likely that activation and inactivation of several CNS-acting drugs may take place at or close to the target neuronal tissues, adding a new dimension to the kinetics of these drugs. It is possible that CYP2D6 and other forms may participate in the synthesis and degradation of exogenous and endogenous neuroactive compounds (53). Thus, generation of reactive metabolites or metabolic disorders *in situ* may lead to neurotoxicity or neoplasia.

Leukocytes

Because of the abundance and relatively easy availability of human peripheral leukocytes, much effort has been devoted to using them as surrogates for P450 activities in other tissues. Analogous with several other extrahepatic tissues, aryl hydrocarbon hydroxylase (AHH) was the first monooxygenase activity demonstrated in human leukocytes and pulmonary alveolar macrophages (34).

Beginning with the pioneering studies of Kellerman et al. (60), there was a flurry of reports on the association between lymphocyte AHH activity and cigarette smoke-induced lung cancer. Many reports suggested that a relationship exists between higher AHH inducibility in lymphocytes and the occurrence of carcinoma of the lung and other tissues, while other studies did not find such an association (3,7).

Because of differences between monocytes and lymphocytes (61), there is concern about the validity of studies done with unselected leukocyte fractions. Recent studies have also demonstrated that the metabolic activation of benzo[a]pyrene in peripheral leukocytes correlates poorly with that in lung tissue (62). Recent studies using both conventional Northern blotting technique (63) and quantitative RT-PCR approach (35) for lymphocyte CYP1A1 and Ah receptor expression (45) have not resolved these discrepancies. It seems that the expression of CYP1A1 may be controlled distinctly in the lung and lymphocytes, which consequently may not be suitable as a surrogate for lung microsomes concerning the pulmonary activation of PAHs (62,64).

Mammary Gland

As in most other extrahepatic tissues, oxidative metabolism and activation of benzo[a]pyrene were first indications of the presence of P450 in mammary gland. AHH activities in human mammary epithelial cells and mammary tumors range from nondetectable to relatively high levels and are inducible by some aromatic substances (34). In a study involving 188 patients with malignant or benign breast tumors, AHH activity was found to be higher in malignant than benign breast tumors (65). In addition, the survival and the disease-free interval of the cancer patients who had low mammary AHH activity was significantly longer than with patients having high AHH activity, suggesting that AHH activity may reflect the overall malignant potential of breast cancer.

Using a monoclonal antibody against rat CYP1A in an immunohistochemical study, Murray and Burke (25) detected positive staining in the cytoplasm of breast tumor cells, while no staining occurred in adjacent normal breast epithelium. Thus, studies in which either AHH activity or CYP1A protein were used as indicators show that the expression of CYP1A member(s), most probably CYP1A1, is increased in cancerous breast cells.

Current Problems and Future Research Needs

In addition to a paucity of knowledge of tissue-specific expression of different CYPs (Table 2), some further issues have to be addressed here.

First, some of the hepatic P450s seem to be liver specific, i.e., they are not found in extrahepatic tissues. One such form is

CYP1A2, which is the predominant form of the two CYP1A members in the liver, although it is quite variably expressed. CYP1A2 is capable of activating various carcinogens, which give rise to extrahepatic tumors. It is possible that CYP1A2 as an activating enzyme represents a case in which activation occurs in the nontarget tissue and reactive products are transferred to the target tissue.

Another problem is concerned with the significance of low-level expression of mRNA in various tissues, i.e., whether it has any functional or toxicological consequences. Basically here we are faced with an exceedingly sensitive new methodology, RT-PCR. In addition, advances in immunological detection methods have made it possible to show low levels of specific proteins in tissues and here we have the same problem as with RT-PCR: what is the role of these low-level proteins?

Use of isolated hepatocytes has been increasing for mechanistic (and even for toxicological screening) studies. Although these cells tend to lose (or sometimes acquire) properties of importance for the activation of toxicants, nevertheless it is expected that hepatocytes will serve as a useful model in studies of CYP-expression, metabolic activation, and induction in the near future. However, the development of similar approaches for extrahepatic tissues faces tougher challenges.

Finally, we have to consider the future requirements and potential of molecular biological techniques. *In vivo* drug probes, however scientifically appropriate and validated, are cumbersome and expensive, and they carry at least some—however small—risk to the subject. For these reasons it would be useful to have methods that would give the same crucial information with minimal invasiveness. For well characterized and properly validated genetic polymorphisms (a prime example is CYP2D6) this has been possible in the form of DNA-based RFLP and PCR methods (10,37). It would be useful to have similar methods to measure, for instance, the potential inducibility, although necessarily in this case also the exposure should be quantitated. The advances in the understanding of regulation of drug metabolizing enzymes should make it possible to develop such assays in the not too distant future, or at least to answer the question of whether there are people who for genetic reasons have varying responses to inducers or other factors that affect drug metabolism.

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